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Junctional modulation in mouse embryonal carcinoma cells by Fab fragments of rabbit anti-embryonal carcinoma cell serum

(gap and tight junctions/teratocarcinoma antigen/freeze-fracture/electron microscopy)

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ABSTRACT **Mouse embryonal carcinoma PCC4 cells are connected by extensive gap and tight junctions. When the cells are incubated in a medium containing Fab fragments against embryonal carcinoma F9 cells, they round up and a process of junctional removal is initiated. In particular, gap junctions are internalized and after 30 hr of incubation with the anti-F9 Fab fragments both tight and gap junctions are no longer present at the cell surface; however, the cells are still in contact by means of small attachment sites.**

Early stages of embryonic differentiation seem to be dependent upon relative position of individual blastomeres within the cleaving embryo and upon formation of microenvironmental domains (1–3). It is not known how positional information becomes operative. It is likely that developmental patterns are set by the acquisition of cell surface adhesion properties and by the assembly-modulation, at the right time, of intercellular junctions controlling both the metabolic cooperation between cells and the traffic of metabolites and ions along the paracellular routes of permeation (1–6). The formation of specific attachment sites and junctions accompanies the compaction of blastomeres at the eight-cell stage, which in turn triggers future segregation of presumptive cell types (2). The assumption that the surface features of the blastomere are crucial for compaction is also supported by the observation that this morphogenetic event in mouse preimplanted embryo may be prevented by the addition of Fab fragments from rabbit antiserum to embryonal carcinoma F9 cells (7, 8). Anti-F9 Fab fragments can induce reversible decompaction when added to embryos up to the older morulae stage (8), and can block the metabolic coupling in embryonal carcinoma cell lines that are in mutual cooperation (7–9).

The purpose of the present paper is to investigate whether anti-F9 Fab fragments are capable of modulating the junctional assemblies in the PCC4 embryonal carcinoma cell line in which "gap" and "tight" junctions are usually abundant. We have found that the addition of anti-F9 Fab fragments results in the loss of tight junctions and internalization of gap junctions. Further assembly of the same types of junctions is blocked after a 30-hr treatment of the cells with anti-F9 Fab fragments.

MATERIALS AND METHODS

The embryonal carcinoma cell line PCC4/AzaR$_1$ has been described (10). Rabbit anti-F9 serum, rabbit antiserum against embryonic mouse liver cells, and Fab preparations were produced according to the techniques described (8).

Incubation with Anti-F9 Fab Fragments and Control Experiments. PCC4 cells growing in Dulbecco's modified Eagle's medium containing 15% fetal calf serum were suspended in phosphate-buffered saline. After dissociation, the cells were plated on Costar cluster plates in the same culture medium and allowed to grow for either 5 hr or 24 hr to the same final cell density. The anti-F9 Fab fragments (final concentration of 220 μg/ml) were added at these times, and the cells were allowed to grow in the presence of this monovalent fragment for 1 and 5 hr. In some experiments the anti-F9 Fab fragments were added immediately after cell dissociation; the cells were plated and allowed to grow in the presence of anti-F9 Fab fragments for either 1 hr or 30 hr. Control experiments were carried out by using (*i*) untreated cells grown to confluency and (*ii*) rabbit anti-embryonic mouse liver cell Fab fragments added immediately after dissociation. The cells were then plated and grown for 1 hr or 30 hr, always in the presence of the antibody. In other control experiments the medium of 24-hr-old cultures was replaced by phosphate-buffered saline, and the cells were further grown in this Ca^{2+}/Mg^{2+}-free medium for 10 min or 1 hr.

Electron Microscopy. For thin sectioning, the cultures were fixed for 1 hr in 2% glutaraldehyde/0.1 M cacodylate/0.1 M sucrose at pH 7.2 followed by postfixation for 1 hr with 1% osmium tetroxide. The material was dehydrated and embedded in araldite. For freeze-fracture the cultures were frozen in Freon 22 either without fixation or after a short treatment with 2% glutaraldehyde/0.1 M cacodylate/0.1 M sucrose at pH 7.2 followed by infiltration for 1 hr with buffered 25% glycerol. The samples were stored in liquid nitrogen. Balzer apparatus 360 was used. During the whole operation the specimen temperature was kept at −140°C. Thin sections previously stained with uranyl acetate and lead citrate and replicas were examined with a Philips EM 400.

RESULTS

Confluent PCC4 cells untreated or treated with rabbit anti-embryonic mouse liver cell Fab fragments exhibited extensive junctional complexes when examined after thin section or freeze-fracture. Both gap and tight junctions were observed (see below, Figs. 2*C* and 3*A*). After a short period of incubation in a medium containing anti-F9 Fab fragments (Fig. 1*A*) or in a Ca^{2+}/Mg^{2+}-free medium, the PCC4 cells rounded up. Although rabbit anti-mouse embryonic liver serum reacted with the surface of PCC4 cells, incubation of these cells with Fab fragments of such serum for 1 hr and 30 hr did not produce

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Abbreviations: Fab, monovalent antibody binding fragment; PF, protoplasmic fracture face; EF, external fracture face.

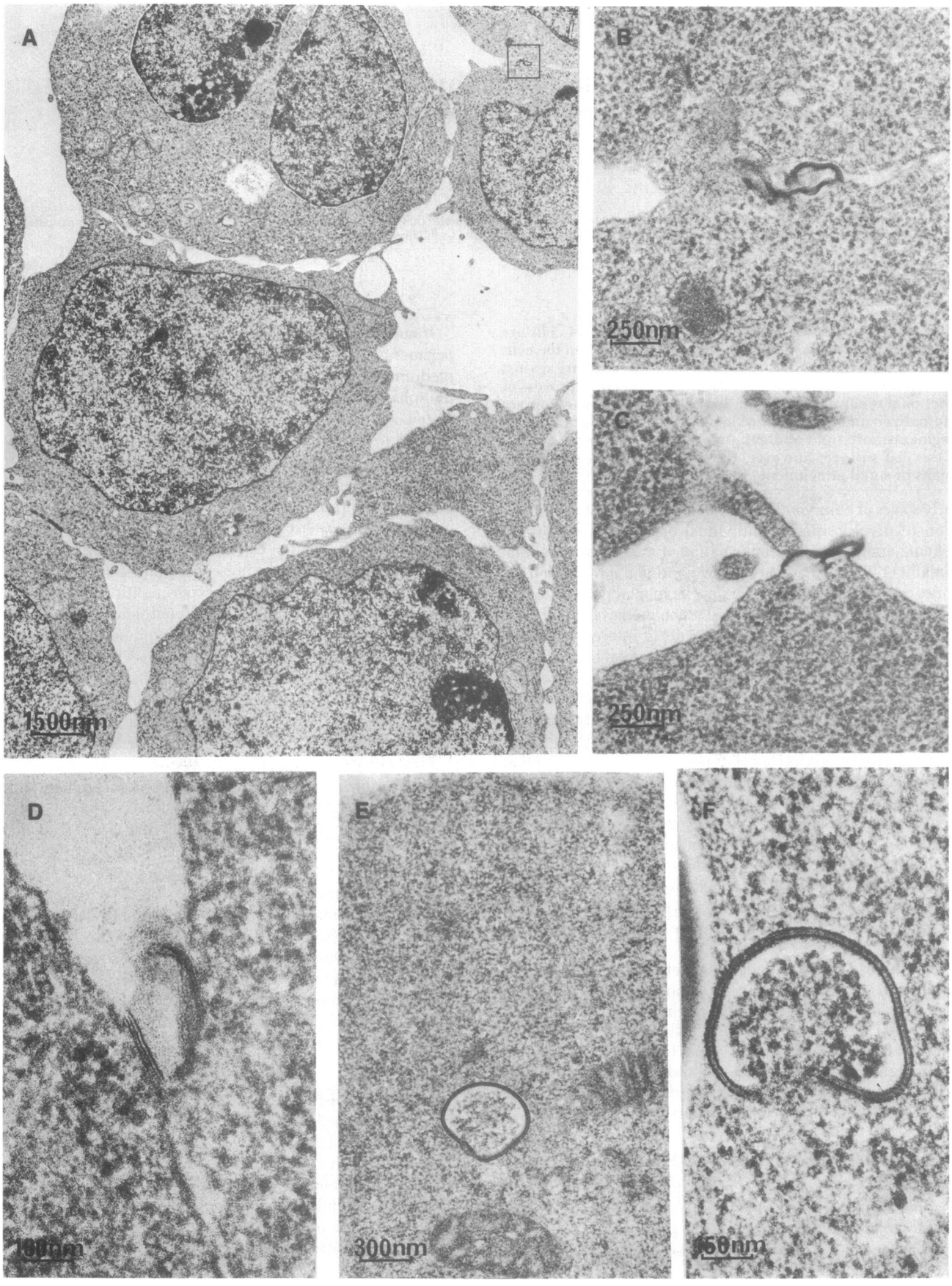

FIG. 1. (*Legend appears at the bottom of the next page.*)

changes either in the cell shape or in the junctional complexes.

Fig. 1 *B*, *C*, and *D* shows a gallery of wrinkled and crescent-shaped gap junctions after 1 hr of incubation with anti-F9 Fab fragments. The gap junctions progressively rounded up and annular profiles developed (Fig. 1*D*). The same cell sometimes displayed superficial junctional blebs as well as annular junctions in deeper cytoplasmic regions (Fig. 1 *E* and *F*). Freeze-fracture of PCC4 samples incubated with anti-F9 Fab fragments during 1–5 hr shows (Fig. 2*A*) small patches and rows of junctional particles dispersed on the protoplasmic fracture face (PF) of the plasma membrane. The external fracture face (EF) had a wrinkled aspect and was marked by pitted images scattered in apparent disorder. In cells incubated in Ca^{2+}/Mg^{2+}free medium for 1 hr (Fig. 2*B*), the intramembranous junctional particles in PF formed a more compact assembly than that visualized in anti-F9-treated samples (Fig. 2*A*) or even in untreated samples (Fig. 2*C*), and the pitted images on EF displayed similar short-range distribution.

Fig. 3*A* shows the tight junction features of untreated PCC4 cells. The general aspect of intricate ridges and furrows characteristic for the tight junction is clearly visible. On the contrary, both in anti-F9 Fab fragment-treated and in phosphate-buffered saline-treated cells (Fig. 3*B*), the ridges appear fragmented and beaded rows of particles are evident, suggesting that the ridges broke up into their constituent subunits. Even the furrows visualized on EF, which in untreated samples appeared smooth (Fig. 3*A*), were occupied by particulate rows (Fig. 3*B*). In thin sections the zonulae occludens inner region, where the outer leaflets of the two adjoining plasma membranes merge in a median line, appears loose (not shown).

Long-term experiments with anti-F9 Fab fragments (30 hr) clearly indicate that the gap and tight junctions were no longer found, although in thin sections the cells were seen to be in mutual contact by means of small attachment sites. Only annular internalized gap junctions in the deepest perinuclear cytoplasmic region were found (Fig. 1*F*). In freeze-fracture replicas long range distribution of intramembranous particles both on plasma membrane PF and EF was mostly random and strongly asymmetric because much more intramembranous particles remained associated with the PF.

DISCUSSION

Despite the recent advances in our understanding of the structure and functional properties of gap and tight junctions, very little is known about their assembly and modulation. Reaggregation experiments show that, after dissociation with medium free of divalent cations, the junctional constituents are still available at the cell surface and can rapidly self-assemble (6, 11–15). Conversely, in differentiating tissues and after cell dissociation by hydrolases, the formation of junctional domains requires the transcription and translation of specific messengers for new junctional proteins (16–18). These studies imply that divalent cations control coupling efficiency of gap junctions and sealing of tight junctions, but that formation of junctions rely upon the presence and stereospecificity of cell surface components (12–15).

In the present contribution we approach another aspect of the junctional assembly; our studies show that the intercellular junctions regulating electrical and metabolic coupling and paracellular routes of permeation can be affected by monovalent antibodies produced against cell surface antigen(s) involved in cell recognition during early embryonic development (7, 8). We have chosen PCC4 cells among various embryonal carcinoma cell lines under investigation because they are characterized by the presence of extensive tight and gap junctions. Treatment of PCC4 cells with the anti-F9 Fab fragments disrupts cell adhesion and triggers a number of steps of cell surface alterations that result in the deletion of gap and tight junctions. The specificity of anti-F9 Fab probably resides in a sequence of events characterized by: (*i*) the occupation of cell surface receptors by the anti-F9 Fab fragments, (*ii*) the cell dissociation followed by changes in cell shape, and finally (*iii*) the removal of tight and gap junctions. The specificity of anti-F9 Fab fragments can also be assessed by our observation that rabbit anti-embryonic mouse liver cell Fab fragments have no effect on cell adhesion, cell shape, and junctional organization. The important question of whether the various morphological aspects observed in anti-F9 Fab fragment-treated cells could be ascribed to a specific signal nature of the interaction between the monovalent ligand with the cell surface is not easily understood. Internalization of gap junctions has been described in various normal and pathological conditions (4, 19–21). Rapid formation of annular gap junctions may follow the specific interaction of regulatory hormones with receptors of the target cell surfaces (17). On the other hand, disassembly and internalization of intercellular junctions have also been observed when cell dissociation is obtained by means of hydrolases (22–24). The effect of anti-F9 Fab fragments should be considered in light of experiments showing that the association of *Dictyostelium* cells is prevented by Fab monovalent fragments directed against specific surface antigens (25) and also of experiments showing that the reassociation of sea urchin blastomeres is prevented by specific Fab fragments against plasma membrane components involved in cell adhesion (26). Conversely, in the mouse embryo during preimplantation, the occupation of receptor sites by divalent crosslink antibody against F9 antigen has no effect on the compaction of blastomeres (8). It is, however, remarkable that only IgG anti-F9, and not divalent antibody against other cell surface components, exhibits a specific competitive effect on the compaction block induced by anti-F9 Fab fragments (8). The complexity of the modulation mechanism of cell surface functions is also illustrated by the observation that antibody against cell surface antigen and multivalent lectins may induce cells to get in mutual contact, but mitogenesis and specific cell surface events—i.e., junctional differentiation—do not necessarily parallel the agglutination. It is only when the ligand is specifically capable of triggering transformation of the target cell that electrical and metabolic coupling develop (27, 28). It is thus tempting to assume that modulation of intercellular junctions results from a specific ligand–cell surface receptor interaction. It is not surprising that the assembly and disassembly of intercellular junctions, which bring to a cell community order, rank, and positional information, do rely upon specific and well-defined variables rather than merely upon crosslinking agents, which may incidentally bring cells together.

FIG. 1 (*on preceding page*). (*A*) Thin section of PCC4 cells grown for 1 hr in the presence of anti-F9 Fab fragments. The rounded cells are held together by wrinkled gap junctions (square) or by small contacts between the tips of microvilli. (*B*) Higher magnification of the area enclosed by a square in *A*. Note the wrinkled aspect of the gap junction. (*C*, *D*, and *E*) Thin sections of PCC4 cells grown for 1 hr in the presence of anti-F9 Fab fragments. *C* shows the tip of a microvillus in touch with a wrinkled gap junction, *D* shows a crescentlike aspect of a gap junction, and *E* shows an annular gap junction found deep inside the cytoplasm. (*F*) Thin section of PCC4 cells grown for 30 hr in the presence of anti-F9 Fab fragments.

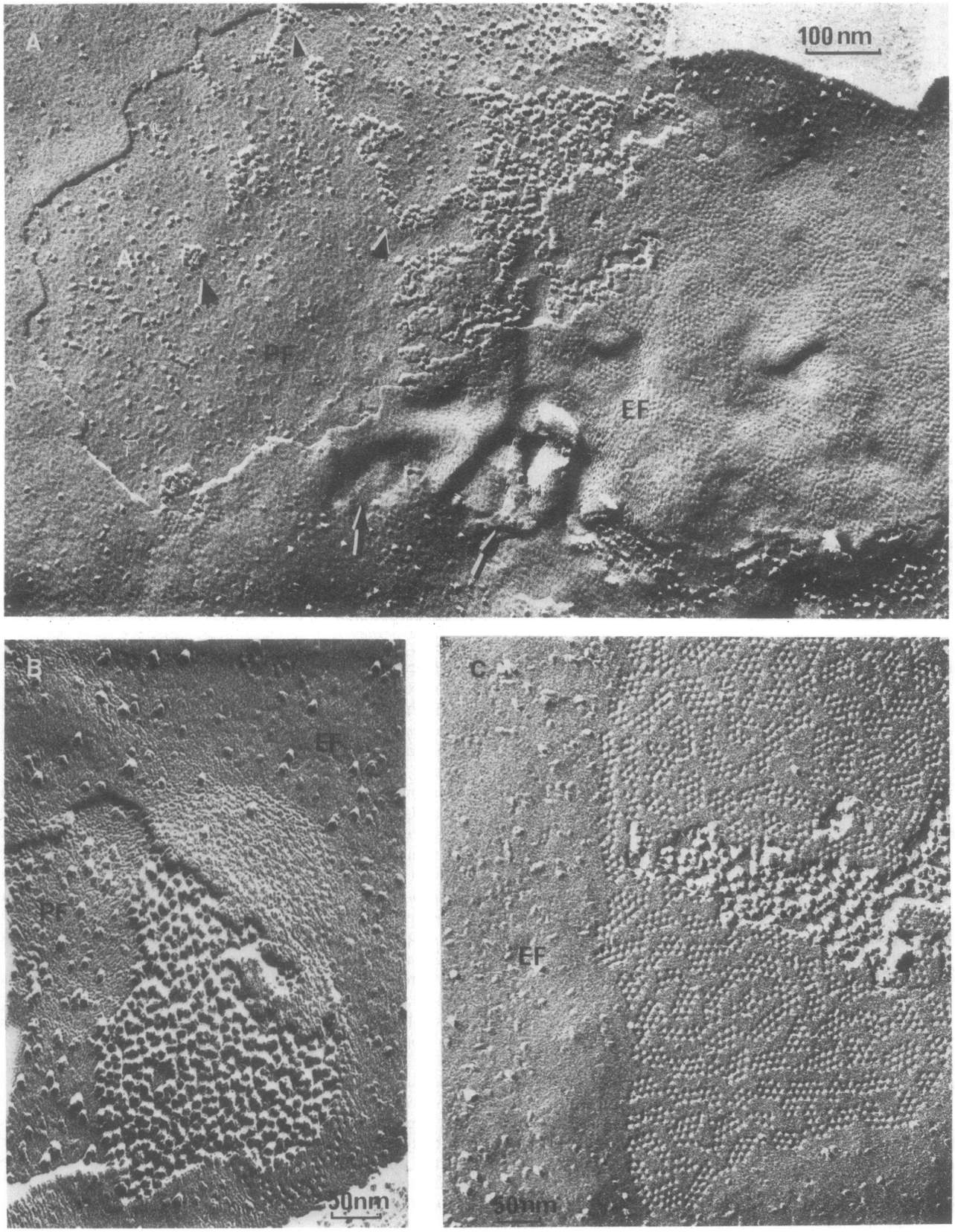

FIG. 2. (*A*) Freeze-fracture image of a gap junction in PCC4 cells grown for 5 hr in the presence of anti-F9 Fab fragments. The junctional particles (arrow heads) and their pitted image are spread over a large area. Fracture faces are characterized by irregular bumps and depressions (arrows). (*B*) Freeze-fracture image of a gap junction found in PCC4 cells grown for 1 hr in presence of Ca^{2+}/Mg^{2+}-free medium. The particulate entities form closely packed arrays. (*C*) Freeze-fracture image of a gap junction in untreated PCC4 cells. Note the geometrically packed rows of pitted images in the EF face separated by pit-free aisles. Arrays of particles are visualized on the PF face.

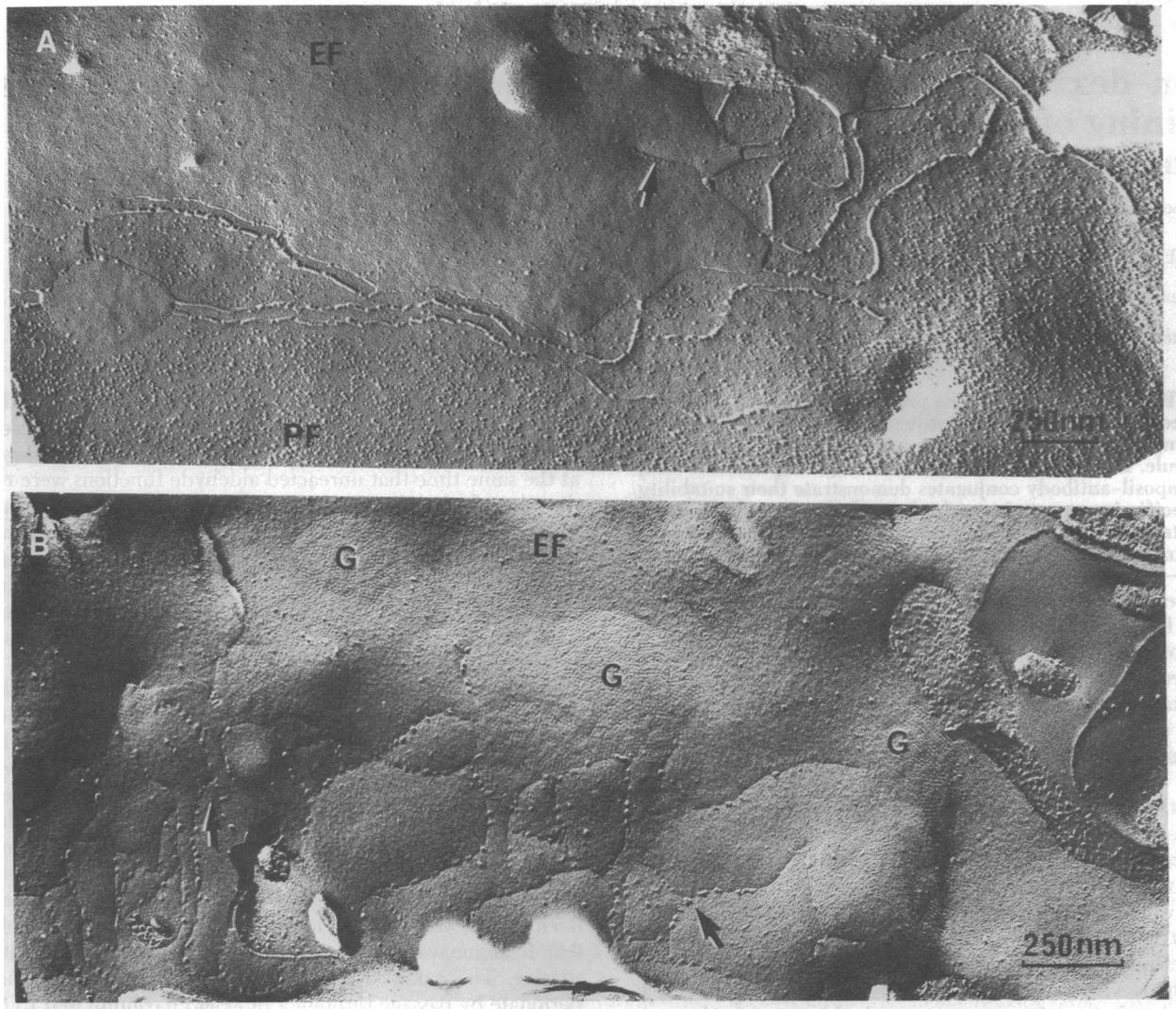

FIG. 3. (*A*) Freeze-fracture aspect of a tight junction in untreated PCC4 cells. Note the intricate pattern of segmented ridges on the PF face. The arrow indicates the smooth appearance of a furrow in the EF face. (*B*) Tight junction in PCC4 cells grown for 1 hr in Ca^{2+}/Mg^{2+}-free medium. The furrows are occupied by beaded fragments (arrow) and arrays of particles. Pitted images of gap junctions (G) are also visible.

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